EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Tobey Tam and Bruce Grant on 05/27/2010.

The application has been amended as follows:

Claims

1-73 (cancelled).

74 (currently amended). A method for determining the sequence ~~in~~ of one or more sequence variations in a target nucleic acid relative to a reference sequence, comprising:

(a) generating mass signals for target nucleic acid fragments ~~and reference nucleic acid fragments~~ by mass spectrometry, wherein the target nucleic acid fragments ~~and the reference nucleic acid fragments~~ result from a specific cleavage reaction of [[a]] the target nucleic acid ~~and a reference nucleic acid into fragments~~;

~~(b) identifying differences in the mass signals between target nucleic acid fragments and reference nucleic acid fragments, thereby identifying different fragments~~

(b) generating or simulating mass signals for reference fragments, wherein the reference fragments result from cleavage or simulated cleavage of the reference sequence using the same specific cleavage reaction in (a);

(c) identifying mass signals in the target nucleic acid fragment spectrum that are different relative to the reference fragment spectrum, thereby identifying different target nucleic acid fragments;

~~(c)~~ (d) generating one or more compomer witnesses corresponding to each different target nucleic acid fragment identified in ~~(b)~~ (c);

~~(d) identifying a reduced set of candidate sequence variations corresponding to the compomer witnesses by determining sequence variations that would lead to the compomer witnesses; and~~

~~(e) determining the sequence in the one or more sequence variations in the target nucleic acid from the reduced set of candidate sequence variations by an algorithm~~

(e) selecting, from the set of all possible subsequences of the reference sequence, a subset of subsequences having at most k cleavage points for the specific cleavage reaction, wherein k is user-defined;

(f) generating for each compomer witness in (d) all possible sequence variations of one or more subsequences in the subset selected in (e) that would lead to the compomer witness, thereby identifying a reduced set of candidate sequence variations; and

(g) scoring the candidate sequence variations identified in (f) to determine the sequence of the one or more sequence variations in the target nucleic acid.

75 (currently amended). The method of claim 74, wherein the differences in mass signals in ~~(b)~~ (c) are additional signals.

76 (cancelled).

77 (previously presented). The method of claim 74, wherein two or more sequence variations are determined.

78 (previously presented). The method of claim 74, wherein the sequence variation is at one or more base positions.

79 (previously presented). The method of claim 74, wherein the sequence variation is a mutation or a polymorphism.

80 (previously presented). The method of claim 79, wherein the mutation is an insertion, a deletion or a substitution.

81 (previously presented). The method of claim 79, wherein the polymorphism is a single nucleotide polymorphism.

82 (previously presented). The method of claim 74, wherein the target nucleic acid is from an organism selected from the group consisting of eukaryotes, prokaryotes and viruses.

83 (previously presented). The method of claim 82, wherein the organism is a bacterium.

84 (currently amended). The method of claim 83, wherein the bacterium is selected from the group consisting of Helicobacter pyloris, Borelia burgdorferi, Legionella pneumophilia, Mycobacteria sp, ~~(e.g.~~ M. tuberculosis, M. avium, M. intracellulare, M. kansaii, M. gordonae ~~)~~, Staphylococcus aureus, Neisseria gonorrheae, Neisseria meningitidis, Listeria monocytogenes, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus sp., Streptococcus faecalis, Streptococcus bovis, Streptococcus pneumoniae, Campylobacter sp., Enterococcus sp., Haemophilus influenzae, Bacillus antracis, Corynebacterium diphtheriae, Corynebacterium sp., Erysipelothrix rhusiopathiae, Clostridium perfringens, Clostridium tetani, Enterobacter aerogenes, Klebsiella pneumoniae, Pasturella multocida, Bacteroides sp.,

Fusobacterium nucleatum, Streptobacillus moniliformis, Treponema pallidium, Treponema pertenue, Leptospira and Actinomyces israelli.

85 (currently amended). The method of claim 74, which further comprises (i) providing a target nucleic acid and a reference nucleic acid; (ii) generating fragments of the target nucleic acid and the reference nucleic acid by specific cleavage; and ~~(iii) determining mass signals of the fragments, wherein the mass signals of (iii) are provided in (a)~~ wherein the fragments of (ii) are provided in (a) and (b).

86 (previously presented). The method of claim 85, wherein the target nucleic acid is in a mixture of nucleic acids.

87 (previously presented). The method of claim 85, wherein the mixture comprises the reference nucleic acid.

88 (previously presented). The method of claim 85, wherein the mixture comprises a plurality of reference nucleic acids.

89 (previously presented). The method of claim 85, wherein the mixture comprises a plurality of target nucleic acids.

90 (previously presented). The method of claim 85, wherein one specific cleavage agent is utilized to generate fragments.

91 (previously presented). The method of claim 85, wherein two or more specific cleavage agents are utilized to generate fragments.

92 (previously presented). The method of claim 85, wherein specific cleavage comprises treatment with an RNAse.

93 (currently amended). The method of claim 85, wherein specific cleavage comprises treatment with a specific cleavage agent selected from the group consisting of RNase T1, RNase U2, the RNase PhyM, RNase A, chicken liver RNase, ~~(~~ RNase CL3 ~~)~~ and ~~cusavitin~~ cusativin.

94 (previously presented). The method of claim 85, wherein specific cleavage comprises treatment with a glycosylase.

95 (previously presented). The method of claim 85, wherein the target nucleic acid is in a pool of nucleic acids from individuals.

96 (previously presented). The method of claim 85, wherein the target nucleic acid is genomic DNA from a single individual.

97 (previously presented). The method of claim 85, wherein the target nucleic acid is selected from the group consisting of single stranded DNA, double stranded DNA, cDNA, single stranded RNA, double stranded RNA, DNA/RNA hybrid, and a DNA/RNA mosaic nucleic acid.

98 (previously presented). The method of claim 85, wherein the target nucleic acid is produced by transcription.

99 -101 (cancelled).

102 (previously presented). The method of claim 74, wherein sequence variations in the target biomolecule permit genotyping a subject, forensic analysis, disease diagnosis or disease prognosis.

103 (previously presented). The method of claim 74, wherein the method determines epigenetic changes in a target nucleic acid molecule relative to a reference nucleic acid molecule.

104 (previously presented). The method of claim 74, wherein the target nucleic acid is from a tumor sample.

105 (currently amended). The method of claim ~~76~~ 74, wherein compomer witnesses are generated based upon parameters selected from the group consisting of mass of the different fragment, peak separation between fragments whose masses differ by a single nucleotide in type or length, and mass spectrometer resolution.

106 (currently amended). The method of claim 74, wherein sequence variations in ~~(d)~~ (f) are determined according to one or more candidate sequences having at most k ~~sequence variations~~ nucleotide insertions, deletions, substitutions and/or modifications compared to the reference sequence.

107 (previously presented). The method of claim 106, wherein k is one or two.

108 (previously presented). The method of claim 106, wherein k is three or more.

109 (cancelled).

110 (currently amended). The method of claim ~~109~~ 74, wherein a simulated spectrum is generated for each sequence variation candidate, and each spectrum is scored.

111 (currently amended). The method of claim ~~109~~ 74, wherein scores assigned to a sequence variation candidate for multiple target nucleic acids are combined for an overall score of the sequence variation candidate.

112 (previously presented). The method of claim 74, wherein sequence variation in the target nucleic acid is recorded in a record.

113 (currently amended). The method of claim 74, wherein the one or more compomer witnesses for each different fragment have a mass within a selected mass difference from the actual mass of the different fragment.

114 (previously presented). The method of claim 113, wherein the mass difference is the resolution of mass measurement.

115 (currently amended). A method for detecting ~~one or more~~ a sequence variation[[s]] in a target nucleic acid, comprising:

(a) generating mass signals for target nucleic acid fragments and reference nucleic acid fragments by mass spectrometry, ~~where~~ wherein the target nucleic acid fragments and the reference nucleic acid fragments result from cleavage of ~~a sample comprising~~ the [[a]] target nucleic acid and reference nucleic acid by ~~multiple~~ two or more specific cleavage reactions, ~~wherein the target nucleic acid is in a nucleic acid mixture, and specific cleavage of a reference nucleic acid by the same cleavage reactions~~;

~~(b) identifying differences in the mass signals between the plurality of fragmentation patterns of target nucleic acid fragments and reference nucleic acid fragments, thereby identifying different fragments;~~

(b) identifying, for at least two of the two or more specific cleavage reactions, mass signals in the target nucleic acid fragment spectrum that are different relative to

the reference fragment spectrum, thereby identifying different target nucleic acid fragments;

(c) identifying different ~~fragments~~ target nucleic acid fragments in each of the at least two specific cleavage reactions that are consistent with ~~a particular~~ the sequence variation in the target nucleic acid, thereby identifying consistent different fragments;

(d) combining the consistent different fragments of (c) to obtain ~~a spectrum~~ set of consistent different fragments;

(e) generating ~~from~~ for each of the ~~spectrum of~~ consistent different fragments of (d) one or more compomer witnesses ~~corresponding to each of the different fragments~~;

(f) determining a reduced set of sequence ~~variations that are candidate sequences~~ variation candidates corresponding to the compomer witnesses; and

~~(g) scoring the candidate sequences of (f); and~~

~~(h) determining one or more sequence variations in the target nucleic acid~~

(g) scoring the sequence variation candidates of (f) to determine the presence or absence of the sequence variation in the target.

116 (cancelled).

117 (previously presented). The method of claim 115, wherein compomer witnesses are generated based upon parameters selected from the group consisting of mass of the different fragment, peak separation between fragments whose masses differ by a single nucleotide in type or length, and mass spectrometer resolution.

118 (currently amended). The method of claim 115, wherein candidate sequence variations in (f) are determined according to one or more candidate sequences having at most k ~~sequence variations~~ nucleotide insertions, deletions, substitutions and/or modifications compared to the reference sequence, wherein k is user-defined.

119 (previously presented). The method of claim 118, wherein k is one or two.

120 (previously presented). The method of claim 118, wherein k is three or more.

121 (previously presented). The method of claim 115, wherein one or more sequence variations are recorded in a record.

122 (currently amended). The method of claim 115, wherein the one or more compomer witnesses for each consistent different fragment have a mass within a selected mass difference from the actual mass of the consistent different fragment.

123 (previously presented). The method of claim 122, wherein the mass difference is the resolution of mass measurement.

124 (currently amended). A method for detecting one or more sequence variations in a target nucleic acid, comprising:

(a) ~~generating mass signals for target nucleic acid fragments and reference nucleic acid fragments by mass spectrometry and~~ providing reference sequence *s* for the target nucleic acid sequence, a description of cleavage reaction conditions, ~~a description of whether or not modified nucleotides are incorporated into all or part of the target sequence, a list of signals corresponding to different fragments between target nucleic acid fragments and reference nucleic acid fragments,~~ and maximal sequence variation order k;

~~(b) generating one or more compomer witnesses c' corresponding to each different fragment identified in (a);~~

~~(c) identifying a reduced set of candidate sequence variations corresponding to the compomer witnesses represented by C_k:={(c[i, j], b[i, j]): $1 \le i \le j \le$ length of s, and ord[i, j] + #b[i, j] $\le$ k}, where C is a set of all bounded compomers within a string s, c [i, j]~~

~~is a compomer corresponding to substring s [i, j], b [i, j] is a boundary of the substring s [i, j]; and~~

~~(d) detecting the one or more sequence variations in the target nucleic acid from the reduced set of candidate sequence variations by an algorithm~~

(b) determining for reference sequence *s* all subsequences s[i,j] in set C_k, wherein s[i,j] represents a subsequence of reference sequence *s* beginning at position i and ending at position j, wherein C_k is described by $C_k:=\{(c[i, j], b[i, j]): 1 \leq i \leq j \leq \text{length of s, and } ord[i, j] + \#b[i, j] \leq k\}$,

wherein c[i,j] represents the compomer corresponding to s[i,j]

wherein b[i,j] represents the boundary corresponding to s[i,j],

wherein ord[i,j] is the number of times s[i,j] is cleaved under the cleavage reaction conditions,

wherein #b[i,j] is the value of b[i,j], wherein:

#b[i,j]=2 if *s* is neither cleaved directly before i nor after j,

#b[i,j]=1 if *s* is cleaved either directly before i or directly after j, but s is not cleaved directly before i and directly after j, and

#b[i,j]=0 if *s* is cleaved directly before i and directly after j;

(c) generating mass signals for target nucleic acid fragments by mass spectrometry, wherein the target nucleic acid fragments result from the cleavage reaction conditions in (a);

(d) generating or simulating mass signals for reference sequence fragments, wherein the reference sequence fragments result from reference sequence s using the cleavage reaction conditions in (a);

(e) identifying mass signals in the target nucleic acid fragment spectrum that are different relative to the reference fragment spectrum, thereby identifying different target nucleic acid fragments;

(f) generating one or more compomer witnesses c' corresponding to each different target nucleic acid fragment identified in (e);

(g) for every compomer witness c', identifying all c[i,j] in C_k such that D(c',c,b) ≤ k, wherein D(c',c,b) is the minimum number of nucleotide insertions, deletions, substitutions or modifications relative to the reference sequence needed to generate the compomer witness c' from c[i,j];

(h) for every compomer c[i,j] identified in (g), determining all sequence variations using at most k-#b insertions, deletions, substitutions or modifications that transform c into c' , thereby identifying a reduced set of candidate sequence variations; and

(i) scoring the reduced set of candidate sequence variations to detect the one or more sequence variations in the target nucleic acid from the reduced set of candidate sequence variations.

Clean copy of claim 124 (note proper use of variables i and j):

124 (currently amended). A method for detecting one or more sequence variations in a target nucleic acid, comprising:

(a) providing reference sequence *s* for the target nucleic acid sequence, a description of cleavage reaction conditions, and maximal sequence variation order k;

(b) determining for reference sequence *s* all subsequences s[i,j] in set C_k, wherein s[i,j] represents a subsequence of reference sequence *s* beginning at position i and ending at position j, wherein C_k is described by C_k:={(c[i, j], b[i, j]): 1 ≤ i ≤ j ≤ length of s, and ord[i, j] + #b[i, j] ≤ k},

wherein c[i,j] represents the compomer corresponding to s[i,j]

wherein b[i,j] represents the boundary corresponding to s[i,j],

wherein ord[i,j] is the number of times s[i,j] is cleaved under the cleavage reaction conditions,

wherein #b[i,j] is the value of b[i,j], wherein:

#b[i,j]=2 if *s* is neither cleaved directly before i nor after j,

#b[i,j]=1 if *s* is cleaved either directly before i or directly after j, but s is not cleaved directly before i and directly after j, and

#b[i,j]=0 if *s* is cleaved directly before i and directly after j;

(c) generating mass signals for target nucleic acid fragments by mass spectrometry, wherein the target nucleic acid fragments result from the cleavage reaction conditions in (a);

(d) generating or simulating mass signals for reference sequence fragments, wherein the reference sequence fragments result from reference sequence s using the cleavage reaction conditions in (a);

(e) identifying mass signals in the target nucleic acid fragment spectrum that are different relative to the reference fragment spectrum, thereby identifying different target nucleic acid fragments;

(f) generating one or more compomer witnesses c' corresponding to each different target nucleic acid fragment identified in (e);

(g) for every compomer witness c', identifying all c[i,j] in C_k such that $D(c',c,b) \leq k$, wherein D(c',c,b) is the minimum number of nucleotide insertions, deletions, substitutions or modifications relative to the reference sequence needed to generate the compomer witness c' from c[i,j];

(h) for every compomer c[i,j] identified in (g), determining all sequence variations using at most k-#b insertions, deletions, substitutions or modifications that transform c into c' , thereby identifying a reduced set of candidate sequence variations; and

(i) scoring the reduced set of candidate sequence variations to detect the one or more sequence variations in the target nucleic acid from the reduced set of candidate sequence variations.

125 (previously presented). The method of claim 124, wherein k is 1 or 2.

126 (previously presented). The method of claim 124, wherein k is 3.

127 (previously presented). The method of claim 124, wherein one or more sequence variations are recorded in a record.

128-129 (cancelled).

130 (currently amended). The method of claim ~~129~~ 124, wherein the differences in mass signals in ~~(b)~~ (e) are additional signals.

131-139 (cancelled).

140 (currently amended). The method of claim ~~129~~ 124, which further comprises (i) providing a target nucleic acid and a reference nucleic acid; (ii) generating fragments of the target nucleic acid and the reference nucleic acid by specific cleavage; and ~~(iii) determining mass signals of the fragments, wherein the mass signals of (iii) are provided in (a)~~ wherein the fragments of (ii) are provided in (c) and (d).

141-157 (cancelled).

158 (currently amended). The method of claim ~~131~~ 124, wherein compomer witnesses are generated based upon parameters selected from the group consisting of mass of the different fragment, peak separation between fragments whose masses differ by a single nucleotide in type or length, and mass spectrometer resolution.

159-163 (cancelled).

164 (currently amended). The method of claim ~~162~~ 124, wherein scores assigned to a sequence variation candidate for multiple target nucleic acids are combined for an overall score of the sequence variation candidate.

165-173 (cancelled).

174 (new). A method of determining a reduced set of sequence variation candidates in a target nucleic acid relative to a reference sequence, comprising:

(a) generating mass signals for target nucleic acid fragments by mass spectrometry, wherein the target nucleic acid fragments result from a specific cleavage reaction of the target nucleic acid;

(b) generating or simulating mass signals for reference fragments, wherein the reference fragments result from cleavage or simulated cleavage of the reference sequence using the same specific cleavage reaction in (a);

(c) identifying mass signals in the target nucleic acid fragment spectrum that are different relative to the reference fragment spectrum, thereby identifying different target nucleic acid fragments;

(d) generating one or more compomer witnesses corresponding to each different target nucleic acid fragment identified in (c);

(e) selecting, from the set of all possible subsequences of the reference sequence, a subset of subsequences having at most k cleavage points for the specific cleavage reaction; and

(f) generating for each compomer witness in (d) all possible sequence variations of one or more subsequences in the subset selected in (e) that would lead to the compomer witness, thereby identifying a reduced set of candidate sequence variations in the target nucleic acid.

175 (new). The method of claim 174, further comprising scoring the candidate sequence variations identified in (f) to determine the sequence of one or more sequence variations in the target nucleic acid.

176 (new). The method of claim 174, wherein the differences in mass signals in (c) are additional signals.

177 (new). The method of claim 174, which further comprises (i) providing a target nucleic acid and a reference nucleic acid; (ii) generating fragments of the target nucleic acid and the reference nucleic acid by specific cleavage, wherein the fragments of (ii) are provided in (a) and (b).

178 (new). The method of claim 177, wherein one specific cleavage agent is utilized to generate fragments.

179 (new). The method of claim 177, wherein two or more specific cleavage agents are utilized to generate fragments.

180 (new). The method of claim 174, wherein compomer witnesses are generated based upon parameters selected from the group consisting of mass of the different fragment, peak separation between fragments whose masses differ by a single nucleotide in type or length, and mass spectrometer resolution.

181 (new). The method of claim 175, wherein the one or more sequence variations are determined according to one or more candidate sequence variations

having at most k nucleotide insertions, deletions, substitutions and/or modifications compared to the reference sequence, wherein k is user-defined.

182 (new). The method of claim 174, wherein a simulated spectrum is generated for each sequence variation candidate, and each spectrum is scored.

183 (new). The method of claim 174, wherein scores assigned to a sequence variation candidate for multiple target nucleic acids are combined for an overall score of the sequence variation candidate.

Additional Matters

1. The provisional double patenting rejection over co-pending application 10/933,611 is withdrawn pursuant to MPEP 804(I)(B)(1) since there are no other rejections in the instant application and the instant application was the earlier filed application.

2. It is noted that the filing receipt (a copy of which was provided with the incoming letter received 07/19/2004) as well as the current Bibliographic Data for this application indicates priority benefit to two provisional applications: 60/429,895 and 60/466,006. However, the declaration received 07/19/2004 as well as the first paragraph of the specification as filed only indicate a claim under 35 USC 119(e) to 60/429,895. The second paragraph of the specification incorporated by reference the disclosure of provisional application 60/466,006, and this may have been the reason for the Office error in originally ascribing priority to the '006 application. The examiner has corrected this error in the Bibliographic Data for the application.

3. The examiner is including with this Office action corrected copies of certain of the previously signed IDS submissions; the corrections were made to provide dates for certain of the references (e.g. GenBank files and Internet web pages) which were not indicated on the original IDS. One reference (A299) on the IDS from 04/18/2006 has been lined through since no date could be determined for this reference. However, the contents of the reference are not considered relevant to the patentability of the claims. Therefore, while the content of the reference has been considered, the reference will not be listed on the patent.

Reasons for Allowance

The amendments above were made to more clearly claim the invention, correct minor typographical errors and inconsistencies, and cancel claims that were substantially duplicates of claims as amended above. The closest prior art is either Zabeau (WO 00/66771) or Foote (WO 98/54571), both references of record. However, neither Zabeau nor Foote teach or suggest the aspect of identifying a reduced set of sequence variation candidates (reflected in steps e and f of claim 74, step f of claim 115, steps b-h of claim 124, and steps e and f of claim 174) from which the sequence is determined. Rather, these references relied on complementary cleavage reactions to arrive at the sequence. Hence, these prior art methods did not involve selection of only certain portions of the reference sequence from which to generate sequence variation candidates, nor scoring said sequence variation candidates to arrive at the sequence.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably

accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL C. WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/
Examiner, Art Unit 1637